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Aspergillus nidulans wetA Activates Spore-Specific Gene Expression

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The *Aspergillus nidulans wetA* gene is required for synthesis of cell wall layers that make asexual spores (conidia) impermeable. In *wetA* mutant strains, conidia take up water and autolyze rather than undergoing the final stages of maturation. *wetA* is activated during conidiogenesis by sequential expression of the *brlA* and *abaA* regulatory genes. To determine whether *wetA* regulates expression of other sporulation-specific genes, its coding region was fused to a nutritionally regulated promoter that permits gene activation in vegetative cells (hyphae) under conditions that suppress conidiation. Expression of *wetA* in hyphae inhibited growth and caused excessive branching. It did not lead to activation of *brlA* or *abaA* but did cause accumulation of transcripts from genes that are normally expressed specifically during the late stages of conidiation and whose mRNAs are stored in mature spores. Thus, *wetA* directly or indirectly regulates expression of some spore-specific genes. At least one gene (*wA*), whose mRNA does not occur in spores but rather accumulates in the sporogenous phialide cells, was activated by *wetA*, suggesting that *wetA* may have a regulatory function in these cells as well as in spores. We propose that *wetA* is responsible for activating a set of genes whose products make up the final two conidial wall layers or direct their assembly and through this activity is responsible for acquisition of spore dormancy.

Most members of the class *Euascomycetes*, including the familiar genetic model fungi *Aspergillus nidulans* and *Neurospora crassa*, and their imperfect (entirely asexual) relatives, such as *Penicillium* spp., abundantly produce mitotically derived spores, called conidia. The morphologies of the sporophores, called conidiophores, vary according to species and are the primary basis of classification of the Fungi Imperfecti, or *Deuteromycetes*, a form-class that includes many medically and economically significant species that are not known to reproduce sexually. Some conidiophores, such as that of *N. crassa*, are structurally fairly simple, consisting of modified hyphae that bud off spores (37). Other conidiophores, such as those of *A. nidulans*, are more complex, consisting of several differentiated cell types, one of which (the phialide) repeatedly produces spores by a specialized budding process (29, 32). The mechanisms controlling conidiophore development and spore differentiation in *A. nidulans* and *N. crassa* are accessible to investigation by both classical and molecular genetic approaches (8, 9, 42). Such investigations have shown that conidiation involves the sequential activation of numerous genes, raising at least two questions: What are the biological functions of the products of these many genes, and how is their expression controlled in space and time? Answers to these questions are expected to contribute to our understanding of the processes controlling morphogenesis and the evolutionary relationships of members of this important group of fungi.

Several lines of evidence have implicated three *A. nidulans* genes, *brlA*, *abaA*, and *wetA*, as pivotal regulators of conidiophore development and conidium maturation. First, recessive loss-of-function mutations in any one of these genes result in formation of morphologically abnormal conidiophores that fail to produce mature, dormant conidia (10, 11, 13, 26). Second, each mutant fails to accumulate most sporulation-specific mRNAs but is unaffected in accumulation of non-developmentally regulated mRNAs (10, 13, 38, 44). Third, ectopic expression of *brlA* or *abaA* in vegetative cells (hyphae) under conditions that normally suppress conidiation leads to (i) cessation of growth, (ii) cellular differentiation events reminiscent of those occurring during normal conidiogenesis, (iii) activation of most conidiation-specific genes, (iv) posttranscriptional inhibition of expression of genes encoding catabolic functions, and (v) rapid turnover of protein and RNA (1, 2, 30).

On the basis of these results and the epistatic relationships of *brlA*, *abaA*, and *wetA* (25), Mirabito et al. (30) proposed a regulatory model describing how the interactions of the products of these genes controlled the temporal and spatial specificity of gene expression during development. In this model, *brlA* activates *abaA*, which in turn acts as a positive-feedback regulator of *brlA* and activates *wetA*. *brlA* and *abaA* independently activate class A genes, which are turned on early during development. *abaA* and *wetA* interact to induce expression of class B genes, which are turned on late during development and code for mRNAs that accumulate in mature conidia. The products of all three genes are required to activate class C and D genes, which were proposed to be expressed specifically in the sporogenous phialide cells. It was further suggested that *wetA* is positively autoregulatory, because a temperature-sensitive mutation in *wetA* prevented *wetA* mRNA accumulation.

Of these three regulatory genes, only one, *wetA*, is active in differentiating conidia. Although *brlA* and *abaA* are active in phialides, as determined by β-galactosidase production in strains containing *brlA*- or *abaA-lacZ* fusion genes (4), and the phialide and conidial cytoplasms are initially continuous (29), *brlA* and *abaA* mRNAs are not present in mature conidia (10). By contrast, *wetA* mRNA accumulates preferentially in mature conidia (10), as does β-galactosidase in strains containing *wetA-lacZ* fusion genes (24a). These findings brought into question the proposal that the products of *abaA* and *wetA* were required together for activation of spore-specific (class B) genes (30), because the *abaA* product may not accumulate in differentiating spores. We have therefore tested the hypothesis that *wetA* alone is sufficient for activation of spore-specific genes. In this report, we show that (i) *wetA* encodes a 60-kDa polypeptide, (ii) forced expression of *wetA* in hyphae inhibits growth but does not induce sporulation, (iii) *wetA* activation does not induce

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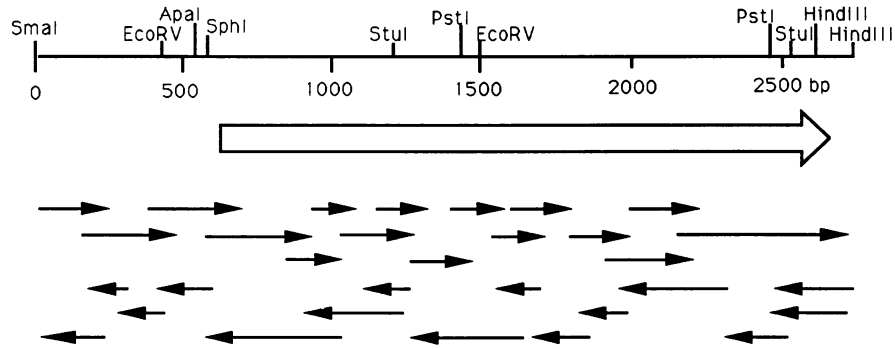

FIG. 1. Restriction map of the *wetA* genomic region and sequencing strategy. A partial restriction map is shown at the top. The position of the *wetA* transcription unit and the direction of transcription are indicated by the large arrow below the restriction map. The starting and ending points and directions of sequences obtained from individual reactions are indicated by the small arrows below the restriction map.

expression of *brlA* or *abaA*, and (iv) *wetA* expression alone is sufficient for class B gene activation, as assayed by mRNA accumulation. On the basis of these results and those from ultrastructural analysis of conidial differentiation in wild-type and *wetA* mutant strains (36), we propose that *wetA* is responsible for activating expression of a set of genes whose products make up the final two conidial wall layers or direct their assembly. Formation of these wall layers appears to be required for, and could be causally related to, acquisition of spore dormancy.

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MATERIALS AND METHODS

***Aspergillus* strains, growth conditions, and genetics.** The following *A. nidulans* strains were used: PW1 (*biA1*; *argB2*; *metG1*; *veA1*), TTAARG (*biA1*; *argB2*/*argB*$^+$; *metG1*; veA1), TMM3 [*biA1*; *argB2*/*argB*$^+$; *alcA*(p)::*wetA*; *metG1*; *veA1*], and FGSC26 (*biA1*; *veA1*). Strains were grown and induced as described previously (1, 20, 22, 30). Standard *A. nidulans* molecular genetic procedures were used (12, 34, 39, 40, 42, 43). Cultures were grown, induced, and harvested as described previously (30).

Construction of pMM11. Plasmid pMM11, containing the *alcA* promoter-*wetA* fusion gene [*alcA*(p)::*wetA*], was constructed by using standard procedures (7). First, a 2,727-bp *Eco*RI (−569)-*Hin*dIII (+2158) fragment was ligated into Bluescript KS+ (Stratagene, Inc., La Jolla, Calif.) to make pMM1. Second, a 540-bp *Sma*I-*Bgl*II fragment from pALCA1 (from D. Gwynne, Allelix Corp.), containing the transcription initiation sites and upstream regulatory sequences of *alcA* (16), was gel isolated and cloned into the *Sma*I and *Bam*HI sites of pMM1 to yield pMM4. Third, the oligonucleotide 5′-CAACCAACAATCAACAGTTGTTCA ATTGTCAATCG-3′ was used to direct the deletion of intervening sequences by using the procedures of Kunkel (21) to yield pMM6. Fourth, the *alcA*(p)::*wetA* fusion gene was cloned as a *Hin*dIII-*Xba*I fragment into Bluescript KS− to yield pMM10. Finally, the 4.1-kb *argB*::chloramphenicol acetyltransferase fusion gene (18) was gel isolated as an *Spe*I-*Not*I fragment from pTA29 (1) and inserted into pMM10 at the *Spe*I and *Not*I sites to yield pMM11. The *alcA*(p)::*wetA* junction and the *wetA* coding region of pMM11 were sequenced to check for undesired mutations. Transformant TMM3, containing a single copy of pMM11 integrated at *argB*, was identified by Southern blot analysis.

Nucleic acid sequencing, RNA mapping, and protein sequence comparisons. *wetA* was cloned both as a 2.7-kb *Sma*I-*Xba*I fragment and as a *Hin*dIII-*Eco*RI fragment into Bluescript KS+ to provide single-stranded DNA templates of both strands for single-stranded sequencing. Overlapping clones were generated by exonuclease III digestion followed by S1 nuclease digestion (15, 19) and were recircularized by ligation. Single-stranded templates were sequenced by using Sequenase (United States Biochemical Corp., Cleveland, Ohio). Complete sequence was obtained from both DNA strands. The 5′ and 3′ ends of the *wetA* gene were determined by S1 nuclease protection analysis as described previously (1). The predicted WetA polypeptide sequence was compared with translated sequences from the GenBank and EMBL data bases and with protein sequences in the NBRF/PIR data base by using GCG (University of Wisconsin) and IntelliGenetics software.

Nucleic acid isolation, blotting, and hybridization. DNA and RNA were isolated, fractionated by electrophoresis in nondenaturing (DNA) or denaturing (RNA) gels, and transferred to nylon membranes (Hybond-N; Amersham Corp., Arlington Heights, Ill.) as described previously (30, 39). Probes were radiolabeled by nick translation (7) as follows: *wetA*, 2.7-kb *Hin*dIII-*Eco*RI fragment from pMM1; *brlA*, 1.9-kb *Hin*dIII-*Sal*I fragment from pTA39 (1); *abaA*, 2.4-kb *Sal*I-*Bam*HI fragment from pPM11 (30); pCAN clones, entire plasmids; *yA*, 1.5-kb *Bam*HI fragment from pRA83AP (31); *wA*, 0.9-kb *Sal*I-*Xho*I fragment from pNK15 (27); SpoC1, entire plasmid pANSpoC1 (41); and *argB*, 1.8-kb *Sph*I-*Sal*I fragment from pSal*argB* (28a).

Nucleotide sequence accession number. The GenBank accession number for the sequence reported is M35758.

RESULTS

***wetA* encodes a 60-kDa polypeptide.** The *wetA* gene was cloned and partially characterized by Boylan et al. (10), who showed that it consists of a single exon. Figure 1 shows a restriction map of the *wetA* chromosomal region and the position of the *wetA* transcription unit as determined by S1 nuclease protection studies (24a). The DNA sequence of this region (Fig. 2) was determined from both strands by using the sequencing strategy shown in Fig. 1. S1 nuclease protection analysis demonstrated the existence of three equiv-

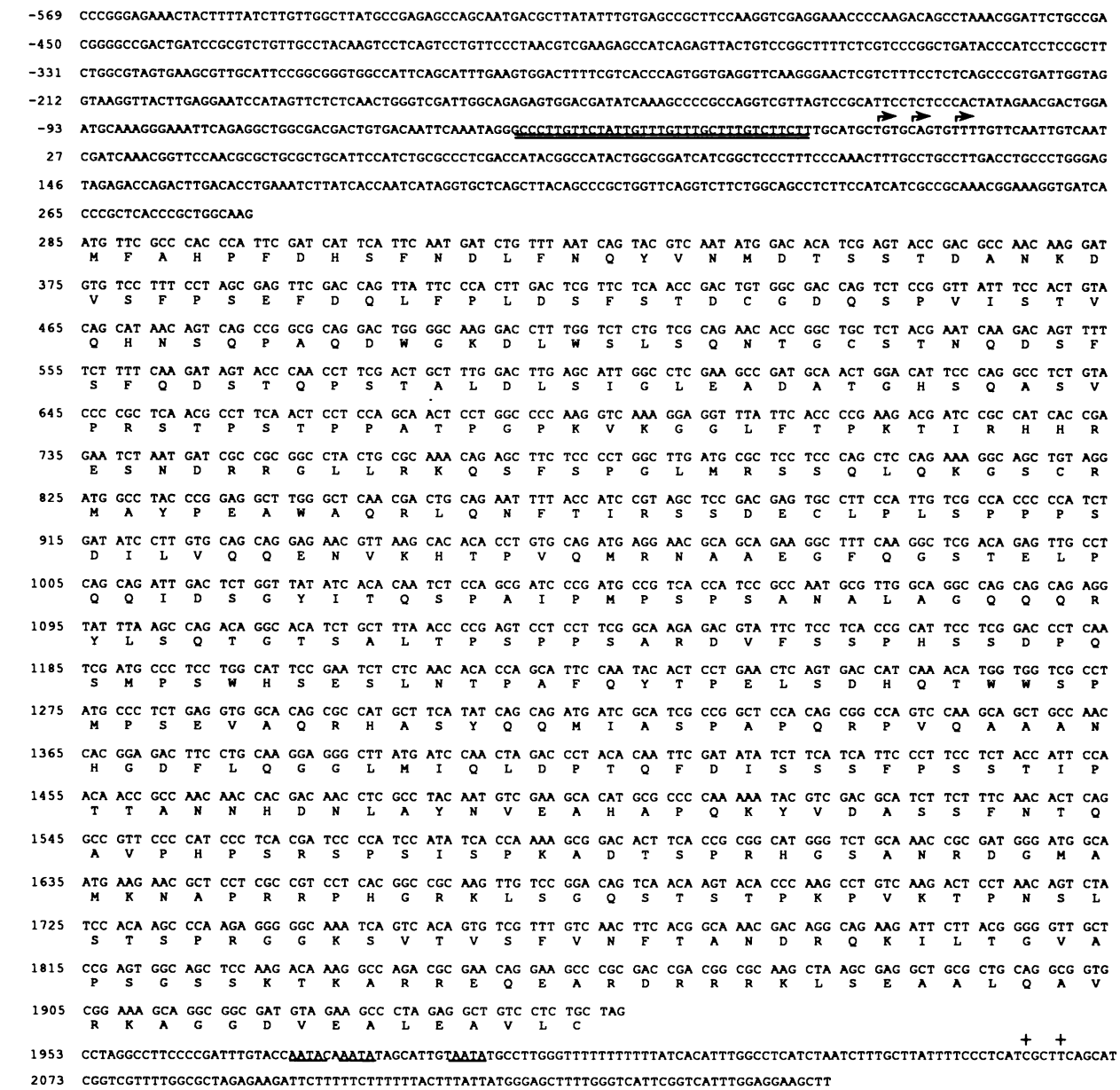

FIG. 2. Nucleotide and predicted amino acid sequences of *wetA*. The *Sma*I-*Hin*dIII region shown in Fig. 1 was sequenced on both strands. Three major transcription initiation sites (⌜*) were mapped by S1 nuclease protection of a 438-nucleotide probe having its 5′ end at nucleotide 284. Two polyadenylation sites (+) were mapped by S1 protection of a 469-nucleotide probe having its 5′ end at nucleotide 2069. Potential polyadenylation signals are singly underlined, and a pyrimidine-rich region preceding the transcription start sites is doubly underlined. A single long translational reading frame beginning with ATG extending from nucleotides 285 to 1950 was identified and is presumed to correspond to the *wetA* coding region. The predicted sequence of the WetA polypeptide (one-letter code) is given below the nucleotide sequence.

alent mRNA cap sites at nucleotide positions +1, +5, and +10 and two equivalent polyadenylation sites at +2062 and +2066, consistent with the approximate mRNA size estimated from denaturing agarose gels (1,800 nucleotides [10]). The transcription initiation sites are preceded by a pyrimidine-rich region similar to sequences found in analogous positions in some other fungal promoters (3, 18), whereas the polyadenylation sites are preceded by three closely spaced, putative polyadenylation signals, AATAN. This region contains one long open translation reading frame, beginning with the first ATG downstream of the mRNA cap sites, at +285, and ending with an amber codon at +1950. The predicted WetA polypeptide is 555 amino acid residues in length (60,209 kDa) and is rich in Ser (14%), Thr (7%), and Pro (10%), similar to the BrlA and AbaA polypeptides (1, 30). Like *brlA* and *abaA* mRNAs, *wetA* mRNA has an unusually long 5′ untranslated region. The WetA polypeptide is slightly basic, with a predicted charge of +1 at pH 7. The carboxy terminus is particularly basic, with 21 of the last 100 residues being Lys, Arg, or His.

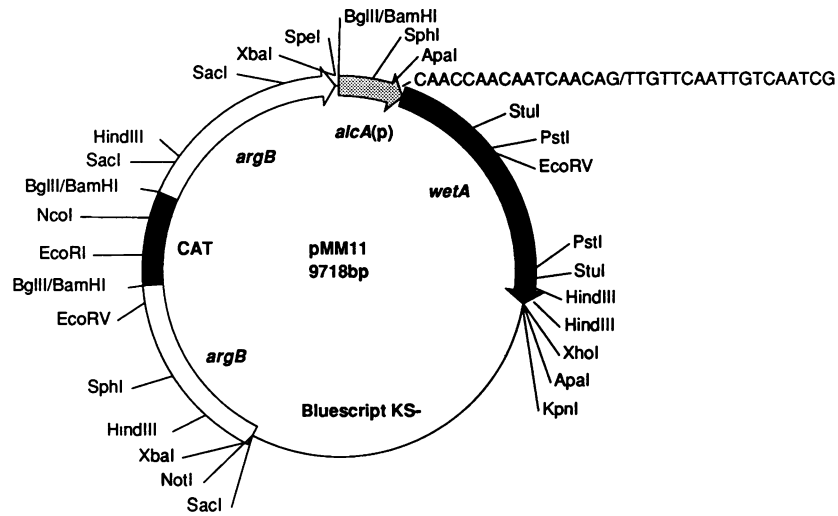

FIG. 3. Structure of the *alcA*(p)::*wetA* fusion gene. Plasmid pMM11 was constructed as described in Materials and Methods. The components of the plasmid are indicated, and the sequence of the mRNA-like strand at the junction of the *alcA* promoter and the *wetA* gene is given. CAT, Chloramphenicol acetyltransferase.

The WetA polypeptide sequence was compared with sequences in the GenBank, EMBL, and NBRF data bases. The highest degree of sequence similarity observed was with the *wee1*-encoded polypeptide of *Schizosaccharomyces pombe* (35), which had 27% amino acid sequence identity over the following regions: WetA, 1 to 546; and Wee1, 115 to 685. However, the best alignment required extensive gaps and involved only intermittent matches. In addition, WetA does not contain a consensus ATP-binding site or a protein kinase catalytic domain analogous to those present in Wee1. We therefore consider it unlikely that the two polypeptides are significantly related. No other significant similarities were found. However, WetA contains several potential $p34^{cdc2}$ phosphorylation sites (23) near its carboxy terminus (Ser/Thr-Pro-Arg/Lys; amino acids 432 to 434, 438 to 440, 470 to 473, and 483 to 485).

Expression of *wetA* in hyphae inhibits vegetative growth. The *wetA* gene is activated only during conidiophore development, and its mRNA accumulates preferentially in mature conidia. To bring *wetA* expression under convenient experimental control, the gene was fused with the *A. nidulans alcA* promoter (Fig. 3) to yield the *alcA*(p)::*wetA* fusion gene (1, 16, 30). *alcA* transcription is induced in hyphae by threonine and repressed by glucose (24). The *alcA*(p)::*wetA* fusion gene in plasmid pMM11 (Fig. 3) was transformed into the *A. nidulans* genome by forced homologous recombination at the *argB* locus (18, 43). Figure 4 shows the phenotypes of random arginine-independent transformants plated on media containing glucose or threonine as the sole carbon source. With glucose as the carbon source, all transformants had growth rates and forms equivalent to those of the recipient strain, PW1. By contrast, approximately half of the transformants showed reduced growth and sporulated poorly on medium containing threonine as the carbon source. Southern blot analysis showed that all transformants displaying reduced growth on threonine possessed integrated copies of pMM11, whereas none of the transformants displaying normal growth contained plasmid DNA sequences (data not shown). This latter class of transformants presumably arose by nonintegrative repair (gene conversion) of the *argB2* allele of PW1 (43). One transformant, designated TMM3, containing a single integrated copy of pMM11, was selected for further analysis. TMM3 grew slowly, branched excessively, and sporulated poorly on media containing threonine as the carbon source (data not shown).

To confirm that the *wetA* gene was transcribed from the *alcA* promoter as predicted, vegetative cultures of TMM3 were grown for 12 h in medium containing glucose as the sole carbon source, and the cells were harvested, washed, and resuspended in medium containing either glucose or threonine as the carbon source. As a control, an isogenic strain (TTAARG [1]) containing a single integrated copy of a plasmid similar to pMM11 but lacking the *alcA*(p)::*wetA* fusion gene was treated identically. In addition, a nearly wild-type strain (FGSC26) was induced to conidiate by filtration and aeration (22). RNA was isolated at various times, and samples were fractionated electrophoretically, blotted, and hybridized with a *wetA*- or *argB*-specific probe. Threonine induction caused accumulation of *wetA* mRNA in hyphae within 1 h, and *wetA* mRNA was present in the cells for at least 6 h (Fig. 5). The transcript level was higher than in FGSC26 cultures that had been induced to conidiate for 30 h by conventional methods. Such cultures are heterogeneous, containing hyphae, conidiophores, and conidia. This cellular heterogeneity is expected to dilute out *wetA* mRNA, which accumulates preferentially in conidia (10).

***wetA* fails to activate *brlA* or *abaA*.** It has been demonstrated that *brlA* activates *abaA*, which in turn activates *wetA* (1). In addition, *abaA* is a positive-feedback regulator of *brlA* (30). To determine whether *wetA* induction led to activation of *brlA* or *abaA*, RNA was isolated from threonine-induced and control cultures of TMM3 and TTAARG, and gel blots were hybridized with a *brlA*- or *abaA*-specific probe. *brlA* and *abaA* transcripts were undetected in induced TMM3 cells (Fig. 6). By contrast, *wetA* mRNA accumulated as expected, and similar amounts of *argB* mRNA were detected in all gel lanes except that with mRNA from strain TMM3, 6 h postinduction, which had a significantly lower level. Reduced *argB* mRNA levels have also been observed following *alcA*(p)-induced expression of *brlA* and *abaA* (30). However, it is unlikely that these reduced *argB* mRNA levels affected the ability of strains to grow in

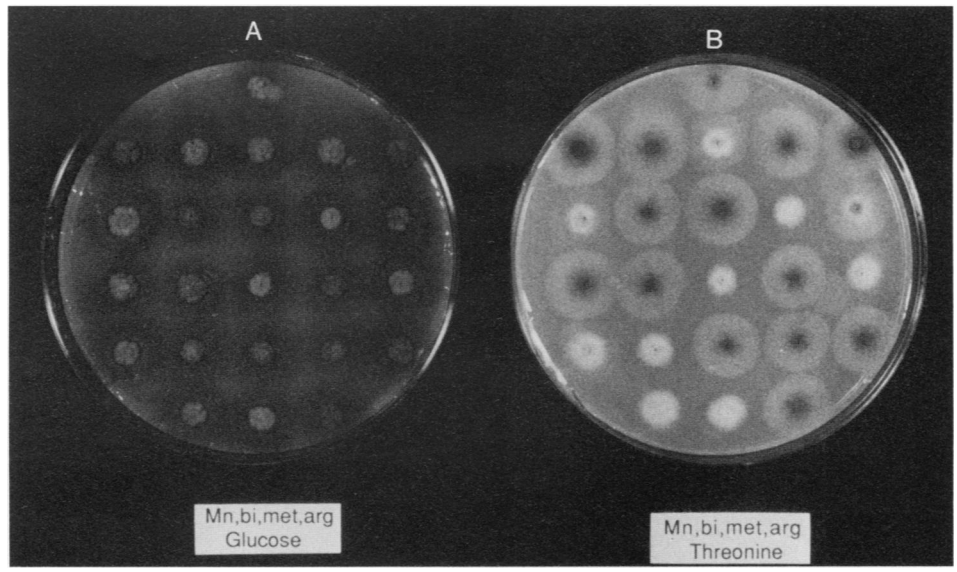

FIG. 4. Inhibition of vegetative growth by genomic incorporation of pMM11. Arginine-independent colonies obtained by transformation of *A. nidulans* PW1 with pMM11 were replica plated on minimal medium (Mn) supplemented with biotin (bi), methionine (met), and arginine (arg) containing glucose (A) or threonine (B) as the sole carbon source. The colony at the top of each plate is the recipient strain. Southern blot analysis demonstrated that strains showing slow growth and abnormal morphology contained one or more integrated copies of pMM11, whereas strains showing normal growth and morphology lacked integrated plasmid and presumably arose by gene conversion.

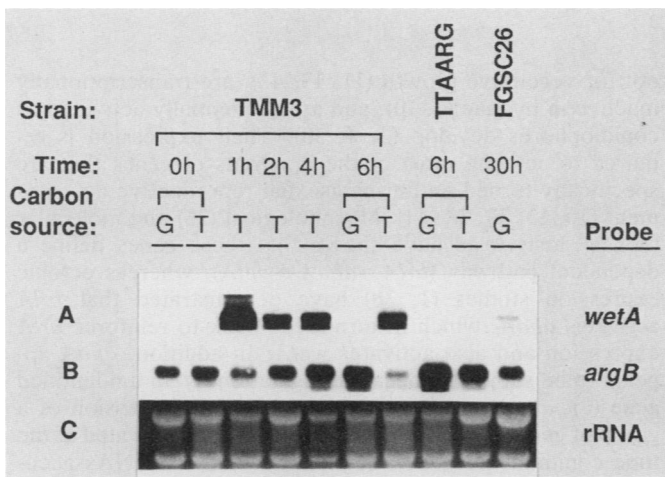

FIG. 5. Transcription of *wetA* from the *alcA* promoter. *A. nidulans* TMM3, containing a single integrated copy of pMM11, and TTAARG, containing a single integrated copy of a plasmid identical to pMM11 but lacking the *alcA*(p)::*wetA* fusion gene, were grown for 12 h in submerged culture with glucose as the carbon source. The cells were harvested, washed with medium containing threonine as the carbon source, and then suspended in medium containing either glucose, a repressive carbon source (lanes G), or threonine, an inductive carbon source (lanes T). At the times indicated, samples of the cultures were harvested and RNA was extracted. RNA was also extracted from a nontransformed strain (FGSC26) that had been grown and induced to conidiate by exposure of hyphae to air for 30 h (22). This culture contained fully mature conidiophores, conidia, and hyphae. RNA samples were fractionated by denaturing gel electrophoresis and transferred to nylon membranes. These were hybridized with gene-specific probes for *wetA* (A) and for *argB* (as a control for gel loading) (B). A parallel gel was stained with ethidium bromide and photographed (C) as an additional control for gel loading.

the absence of arginine, because extremely low *argB* mRNA levels have been shown to be adequate for prototrophic growth (28). Thus, *alcA*-directed activation of *wetA* does not cause *brlA* or *abaA* transcript accumulation.

***wetA* activates spore-specific gene expression.** To determine whether *wetA* induction leads to activation of other sporulation-specific genes, RNA isolated from threonine-induced

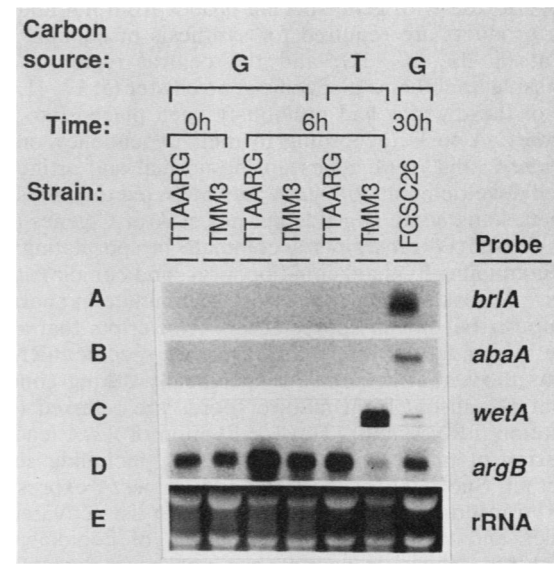

FIG. 6. Failure of *wetA* to activate *brlA* or *abaA*. *A. nidulans* TMM3 and TTAARG were grown and induced for 6 h as described in the legend to Fig. 5, and RNA was isolated. RNA from an FGSC26 culture that had been induced to conidiate for 30 h was also isolated as a positive control. Following gel fractionation, blots were hybridized with gene-specific probes for *brlA* (A), *abaA* (B), *wetA* (C), or *argB* (D). A parallel gel was stained with ethidium bromide and photographed (E).

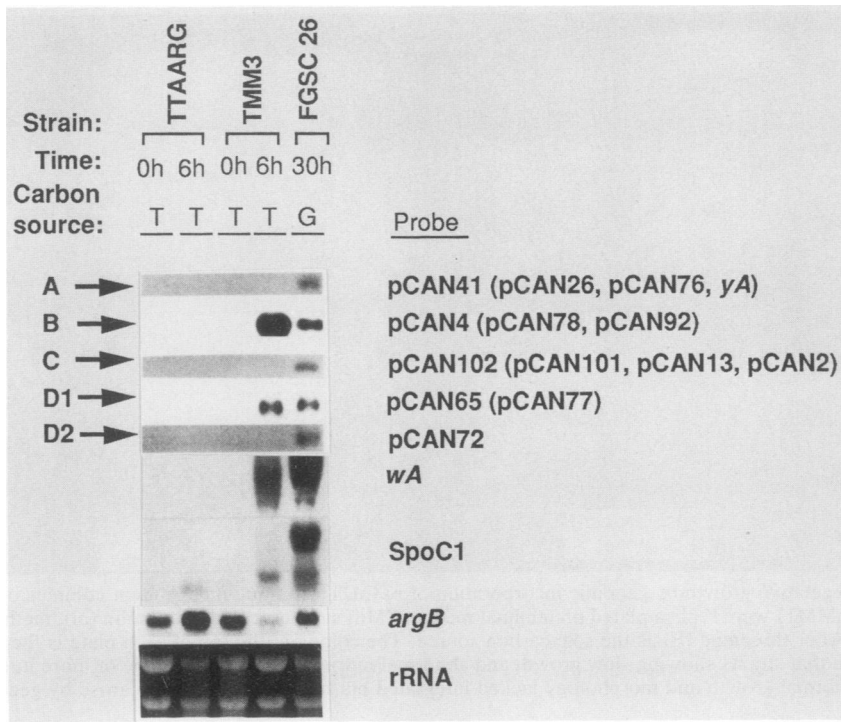

FIG. 7. *wetA*-induced spore-specific gene expression. Strains TMM3, TTAARG, and FGSC26 were treated as described in the legend to Fig. 6, and RNA blots were hybridized with the probes indicated. pCAN clones correspond to mRNAs that accumulate specifically during conidiation (10). Where representative data are shown, additional pCAN clones producing the same hybridization pattern are listed in parentheses.

and control cultures of TMM3 and TTAARG was hybridized with cDNA clones (called pCAN clones, for conidiation in *A. nidulans*) corresponding to mRNAs that accumulate specifically during conidiation (10, 38, 44). Gel blots were also hybridized with gene-specific probes from *wA* and *yA*, whose products are required for synthesis of conidial wall pigment (6, 13, 27, 31), and the central region of the dispensable SpoC1 spore-specific gene cluster (5, 17, 41, 44). Many of these genes had previously been placed into four categories, A to D, according to their dependency on the *brlA*, *abaA*, and *wetA* genes during normal and artificially induced development (30). *wetA* activation led to expression of B genes and some D genes but not of A or C genes (Fig. 7). B, C, and D transcripts accumulate in sporulating cultures (containing hyphae, conidiophores, and conidia) and in spores, whereas most A transcripts accumulate in sporulating cultures but not in spores (29a). Transcripts that accumulate in conidia are referred to as spore-specific mRNAs, whereas those that accumulate specifically during conidiation but are absent from mature spores are referred to as sporulation mRNAs (38). Thus, activation of *wetA* leads to expression of many spore-specific genes, including some, but not all, SpoC1 (17) genes. In addition, *wetA* expression causes transcription of *wA*, a gene that is activated in phialides and is required for formation of conidial wall pigment but whose transcript has not been detected in conidia (27).

DISCUSSION

It has been proposed that *brlA*, *abaA*, and *wetA* comprise the central pathway controlling asexual development in *A. nidulans* (30). These genes are required for conidiation but not for vegetative growth (11, 13, 42), are transcriptionally inactive in hyphae (2, 10), and are sequentially activated as conidiophores develop (3, 4, 30). Their expression is required to activate most of the hundreds of genes that are specifically turned on during asexual reproductive development (10, 13, 33, 38, 44). Morphological (26) and molecular (10) epistasis tests have shown that these genes define a dependent pathway (*brlA*→*abaA*→*wetA*), whereas ectopic expression studies (1, 30) have demonstrated that *brlA* activates *abaA*, which in turn feeds back to reinforce *brlA* expression and also activates *wetA*. In addition, *abaA* appears to be subject to negative regulation by an unidentified gene (3). *abaA* and *wetA* are required for expression of a group of genes, called class B genes, that are activated at the time conidium formation begins and whose mRNAs accumulate in mature, dormant conidia.

Results from the study of Mirabito et al. (30) did not permit determination whether *abaA* and *wetA* were required together for B-gene activation or whether *wetA* alone was sufficient. In this study, we investigated the effects on gene expression of activating *wetA* in vegetative cells under conditions that suppress conidiation. The data showed that *wetA* activation did not cause accumulation of *brlA* or *abaA* mRNA. It was therefore possible to ascertain which conidiation-specific genes were activated by *wetA* in the absence of expression of the other regulatory genes. The results demonstrated that *wetA* expression was sufficient for activation of B genes but not for activation of A or C genes. Unexpectedly, *wetA* expression also led to activation of some D genes, which had previously been shown to require $brlA^+$, $abaA^+$, and $wetA^+$ activities for threonine-induced expression in hyphae but not for normal development (10,

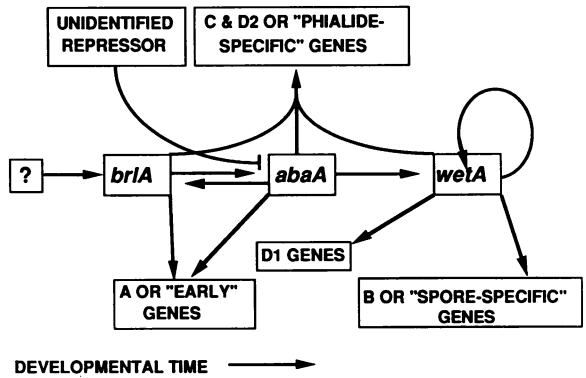

FIG. 8. Model for control of gene expression by *brlA*, *abaA*, and *wetA*. Symbols: →, positive regulation; —|, negative regulation. The classes of responding genes are discussed in the text. Boxed question marks indicate unidentified regulatory elements whose existence has been inferred from previous studies (1, 10, 30).

30). Thus, the previously inferred requirement for $brlA^+$ and $abaA^+$ for expression of some D genes was due to the dependency of *wetA* expression on activation of these earlier regulatory genes. Given that not all D genes are directly activated by *wetA*, we have divided class D into two subclasses, D1 and D2, the former group of genes being directly inducible by *wetA*. Class C and D genes are activated earlier in development than class B genes, but as with class B genes, their mRNAs accumulate in conidia. Given the dependency of class C and many class D genes on expression of all three of the regulatory genes and the observation that there is only one cell type, the phialide, in which all three genes are simultaneously active (4; 24b), it is probable that C and D1 genes are expressed in phialides, even though their mRNAs are also found in mature conidia. At least one phialide-specific gene, *wA* (27), is directly activated by *wetA*, further corroborating that *wetA* is active in phialides. A model for spatial and temporal control of developmental gene expression by *brlA*, *abaA*, and *wetA*, consistent with the available data, is presented in Fig. 8.

Loss-of-function mutations in *wetA* result in formation of defective conidia that autolyze rather than undergoing normal maturation (10, 11, 13), whereas other aspects of conidiophore development are unaffected. Sewall et al. (36) showed that the conidium has four cell wall layers, designated C1 to C4. Two layers, C1 and C2, are produced by the phialide and are unaffected by *wetA* mutations. By contrast, the C3 and C4 layers are formed after the spore becomes separated from the phialide. These wall layers fail to form in *wetA* mutants, a result that in conjunction with molecular data indicates that *wetA* activates genes (B genes) that are responsible for the final stages of spore wall assembly.

Lysis of *wetA* mutant spores indicates that the C3 and C4 wall layers are needed for osmotic stability. It has been observed that *wetA* conidia can be rescued if they are formed in submerged culture in defined medium, which has a moderately high osmotic strength (1). Under these conditions the spores germinate precociously rather than undergoing lysis. Thus, *wetA*-directed functions appear to be required for structural integrity of the spore as well as for acquisition of dormancy.

Given these regulatory functions of *wetA*, it is not surprising that its forced expression in hyphae inhibits growth. The observed excessive branching in strain TMM3 under inductive conditions may be explained by a reduced hyphal extension rate without a coordinated reduction in the rate of side branch formation (14). The reduced growth rate is perhaps predictable in view of the induction by *wetA* of spore-specific genes that presumably encode products involved in synthesis of impermeable cell wall layers. Incorporation of spore cell wall components into walls of vegetative cells is expected to result in a reduction in the ability of these cells to take up nutrients. As *wetA* induction did not lead to activation of genes involved in earlier steps of conidiation, it did not cause spore formation, as does ectopic expression of *brlA* (1).

It has been suggested that *wetA* is positively autoregulatory (30), as indicated in Fig. 8, because *wetA* mRNA fails to accumulate in *wetA6*(Ts) strains during normal (10) or artificially induced (30) asexual development at restrictive temperatures. Autoregulation might be expected given the observations that *wetA* is initially activated by *abaA* but that *abaA* mRNA does not accumulate in conidia, in which *wetA* is presumed to function. Thus, *wetA* may be required to reinforce its own expression once conidia have been delimited from the phialide. It is possible that *wetA* encodes a transcriptional regulator. Alternatively, its product might be involved in stabilizing mRNAs, including its own, that are stored for long periods in the dormant spore. The results presented in this report make it possible to investigate directly the biochemical activities of the *wetA* product.

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